

$$[B + \phi + C + T - P - T1R] \times [B - \phi - C - T + P + T1S]$$

259

12N 1046

Use 1 mol mor: Y41 (679-1831) + Y24 (BdC) into 50 ml colo (Sh. 30°. 12M30.)

12N. 7/1/46. Wash + plate into T/0). Save culture.

	P2	P12	
①	4		
②	5		
③	BCT	7	
④	BφP	14	

{ 7S
2R. to T-1.

A. P12. Pick colonies from 3 and streak out. P13. Test each colonies.
 $T-1 = BC T \cancel{BC} BC - BC = T(0) LR$ probably $(T - P + R)$.

	T-1	R	S
1			
2			
3	R		
4	R		
5	R		
6	S		
7	R		
8	R		
9	R		
10	R		
11	R		
12	R		
13	R		
14	R		
15	R		
16	R		

PTR ✓ parental BCφPT+. See 272

BCφPT+ See 272

more pure or
less toxic.
perhaps should
streak out on
2% agar.

B. P13. Streak out colonies of 1, 2.

	A14 Test		Test P14.		④	P
	T-1	T/0	BP	BφP		
1	S		-	+	-	+
2	S		-	+	+	+
3	R		-	+	-	+
4	S		-	+	-	+
5	S		-	+	-	+
6	S		-	+	-	+
7	S		-	+	-	+
8	R		-	+	-	+
9	R		-	+	-	+
10	S		-	+	-	+
11	R		-	+	-	+
12	R		-	+	-	+

Prolif.

Segregation of Biotinless: Etc.

260.

Check reg.

$T(0)$ ^{round} $T(B)$.

1. 254-144.	+	+
2. 254-146.	+	+
3. 254-52	+	+
4. 254-132	-	+
5. 254-42	+	+

Test other colonies from 254.

254-28.

1. $BH - PT - BMPT + BPT - ?$ form $BM1 \times PT$

By assay: B^4 1. check. Parental.

Recotypes

261.

12 JUL 1946

10P12. Establish broads. in 50ml colis 30°sh.

1. Y9 (LM)
2. Y40
3. Y41
4. 58-163
5. 679-183
6. Y24
7. ~~Y33~~ Y38 } n.g.
8. Y39. } for radiation.
9. 58-183X n.g.

broads and colis. Store in refrigerator 7P13.

brod 50 ml colis:

- (1) 161 + Y41
(2) 183 + Y40.

1. (1) 6
2. (1) B. 82:
3. (2) 9
4. (2) B 19. See infra. Isolate and check for Bug., 11.
5. See 262
Y40 + Y41
x-streak.
2 surface colonies. Will have to repeat procedure.
6. 5×10^{-2}

Six: cross-streaking

262

13 JUL 1946

7P13. Using loopsful from 261, crossstreak on coliccs.

A. 11. Stake 1 2.

TLM

1 Y9 TLM 161 BM
2 183 TD
3 Y41 PTR
4 Y40 BMR

B. 11 Y40 BMR 183 PT
12 Y41 PTR

C 21 Y41 PTR 161 BM. Smear.

D 31 Y38 Y39.

Scrape growth from B11 B12, suspend in H₂O and plate into T/0.

No colonies

13 JUL 1946

Irradiate Y38 and Y39 cultures (see 200) ~~2 min.~~ and inoc. col. as.

11 P.M. Sh. 35°.

Y38 $\frac{1}{1}$ min
 $\frac{2}{5}$ "

Test Y39 -

dl leuc.
dl isole.
l-leuc.Y39 $\frac{1}{1}$ min
 $\frac{2}{5}$ "Inoculation plate Y38 5 min. into T (aer.)
Y39 " into T (Ac + sole). at 10^{-7} . 4 P.M.Y38 - 2 min
Y38 - 1 min 2 plates.

Y38 $\frac{1}{1}$ 600 cells. 4 colonies.
~~5 min~~
 $\frac{2}{2}$
 $\frac{3}{3}$
 $\frac{4}{4}$
 $\frac{5}{5}$
 $\frac{6}{6}$
 $\frac{7}{7}$
 $\frac{8}{8}$
 $\frac{9}{9}$
 $\frac{10}{10}$

1 min $\frac{11}{11}$ 350 cells, 1 colony.
 $\frac{12}{12}$
2 min $\frac{13}{13}$ 500 cells. 9 colonies.
 $\frac{14}{14}$

Y39 $\frac{21}{21}$ did not grow
 $\frac{22}{22}$ evidently ~~soy~~ is not leuc or sole stain.
 $\frac{23}{23}$
 $\frac{24}{24}$
 $\frac{25}{25}$ Test by agar: e.g.: histidine
 $\frac{26}{26}$
 $\frac{27}{27}$
 $\frac{28}{28}$
 $\frac{29}{29}$
 $\frac{30}{30}$
 $\frac{31}{31}$
 $\frac{32}{32}$

See info for tests on Y38 -

Sex: triple cross
TLM x BφC

264.

9P. 7/14/46. 1 ml mix into colloid + c Sh. 30°
Y9 x Y24

N15 Plate ratio:

1	0	0
2	0	0
3	0	0
4	0	0
11	B	0
12	Ø	0
13	C	4
14	T	0
15	L	4
16	M	0
21	BT	0
22	BM	0
23	ØT	0
24	ØL	6. v. sm.
25	ØM	0
26	CT	turbid
27	CL	turbid
28	CM	turbid
31	TLØ	ca. 16
32	TMØ	
	BLT	turbid
	CTL	turbid

very desiccating. (cinnamum? or stramis?)

Throw out plates.

679-680-Y9.

265

Y10.

July 16, 1946.

Check on requirements:

P16: Y9.

TLM	++
TL	-
TM	-
LM	-
TLM+Cyst	++
TL+Cyst	-

Growth is however, not optional at all: methionine; something else may be required. (Consider pair, homocysteine, choline, etc.)

In TL + EAH, NEHA, YE, Vits.

TLM

TL & YE ++. others + or -.

Y10:

TB,	-
LB,	+
TL	-
TLB,	++

probably some $T^+L^-B,-$ in the population. Resists - stands out from TLB.

2/5 isolates tested came up on LB, as well as TLB,
same as Y45. Other three - same 1. as Y10a. (or after
7/27 as Y10.)

Killer E. coli.
Resistance

266

7/15/46.

P 15 more or less Heslby's "T" and "R".

A 16. Filter "T" and test for activity as R in phage.

1.	T + R 1 ml ea.	+++
2.	T 10^{-3} + R	+++
3.	T + R 10^{-3}	++
4.	T 10^3 + R 10^{-3}	++.

no demonstrable killing.

17 JUL 1946

"Reacting" strains "3" and "14" received from Dienes. AT.

Transfus to subculture slants D3 D14.

Stock plates 10A17. D3 swarmed only. Brodes in D14?

Nutritional Requirements: 10P17.

Grows very rapidly complete media

	D3.	9A18	9A19	
Pst Nic Cyst	++	+++		
PN	-	++		
PC	+	+		
NC	++	+++		
Cyst-Vits.	+++	+++±		
				to c. + slowly ++.

	D14	PN	++	+++	
PN	-	-	++		
PC	+	+	+		
NC	++	++	++		
Cyst-Vits.	+++	+++	+++±		
					+ slowly ++.

Repeat for a sp. vits. req.

	Cyst + 10 Bu Vits	D3	D14	10P18. 35°
2		++		
3		++		
4	+	+	+	(nic)
5		++		
6		++		
7		++		
8		++		
9		++	+++	
10.		++	++	

cystine is stimulatory; probably not adaptation.

Concurrent reversion

1 Dec 1968

Recd. from Ryan a "prototroph obtained directly from 679-680. Subculture

1. A17 streak out on T(0). No colonies
2. Mor loopful in T(0) No growth.

P19 - inoc ca 10^7 cells into T(0). Use loopful to mix
 T, L, T_L :

0	+
T	-
L	=
T_L	+++

Not prototroph!

M20. Mor colins \approx 0 Use v. large inoculum. 30° sh.

1DP21. Plate out 1ml \approx into:

1. T(Lc) 10^3
2. T(Hm) 0
3. T(0) 0
4. T(0) 0

267

240-5
size variant (?)

17 JUL 1953

P16. Kroc coli = soned at 30° sl. = 240-5a ♂
K-12. ♂

P17. Dil 10^{-7} and plate in detection plates. T(0)

1. K-12 + A18.
2. 240-5 ~~++~~
3. both. ++

Phage: T-1 susceptible.

Pedigree:

{ 236: ~~58-161~~ \times 679-183 on minimal Dif to H₂O + streak a minimal plate. Pick a colony to water + plate d. (240-1). 2% small colonies. But all prototrophic. Check now for mutability of colony size.

If anything 240-5 is the faster growing colony type
→ 240-5 large colonies at 24h
K-12 small v. indistinguishable.

Repet. June 12 M 18.

Plate 6P19.

1920 - same result as above - K-12 colonies appear more slowly than 240-5 on T(0). They are indistinguishable on ∞ !
[Why was 240-5 first picked up as a small variant?].

TLM x BφC.

210

BφC x TLM

N17 1 ml moi ea. into colis. \rightarrow 30°

530P18. Wash + plate 1 ml $\frac{1}{2}$.

1	0	0	
2	B	0	BL
3	Φ	1?	
4	C	57 T	[C]
5	T	0	[Φ]
6	L	0	
7	M	0	
8	BT	0	
9	BL	20 T	
10	BM	0	<u>average - 264.</u>
11	ΦT	0	
12	TL	0	
13	PM	?2	
14	ZT	T	
15	CL	52 T	
16.	CM	T	

Tay Y18. BφC - TLB;

Six: cross streak.

271

17 JUL 1940

N17. cross streak on colis = 58-161 x 679-183.
1,2

3. streaks = mixed inoculum.

10P19. Plate after T(0). ① @ ca. 10^8
 ② @ ca. 10^9

no dup colonies. ∴ this is not a good lead.

July 18, 1946.
P17. P18¹, 10 P18

A Y	B	$\phi C T P$	$B \phi C T^{-P}$	$B \phi C P^{-T}$	$B \phi P T^{-C}$	$C B C P T^{-\phi}$	$\phi C P T^{-B}$	$B \phi C P T^{-O}$	$\bar{P} T /,$	parental.
A G	G	++	=	=	+	+	+	+	$\bar{D} T /,$	"

C 2	-	$B \phi P$	B	P	B P.	T^{-1}
C 6	-	++	-	++	++	S -

Recombination Types!

See 263.

	Arg.		
1	++		
2	++		✓
3	<u>spreader</u> (not coli)		
4	-	T (A)	
5	-	T	
6			
7	++		
8	-	C - 9 - Methionine. Check in 1.g. Y43 ✓	
9	-	A only. → glutamic. Y49	
10	-	T	
11	-	T	
12	-	T (e)	

256 - 1 T(0) + aux: turbid; A.C. (not coli)
 2 +
 3 +++
 4 +. — ^{ACD} turbid lost.

Y43. T(Arg) T(Meth) T(A.M.)
 - - ++

July 19, 1946.

10P19. Irradiate 24 hour culture Y39 in \approx 2 mins. uv. in medium.

① plate 1 ml in coli \rightarrow $\rho^3 = \underline{2 \text{ to } 3}$.

② drop 1 ml in small colis \approx 63°

Layer N22.

1130P20. Detectes plates - T (hist. line) 10^{-7} and 5×10^{-8}
ca 1200 colonies total. 10 small colonies. pab. 8P23.
to 20 slants. T(H)

1	++	probably not coli
2	-	
3	-	T
4	-	A
5	-	B?
6	-	B -
7	-	B-3 Y44
8	exp. v. sp. on colis.	+ on minimal (E2)
9	+	
10	n.g.	

air xanograph P.25. - Novitamini sp. resp. in 6. checks
in liquid + for yna. B-3 pab.

	12 h.	24 h.	
H	-	-	
" H+pab.	-	+++	
" yna	+	+++	
" M	\pm	\pm	
" M+yna	+++	+++	
-H+pab.	-	-	10F.

Try 1. more pab.
2. pab sterile filtered.
(slow on pab) yna replaces pab.

July 21, 1946.

Broth drop each of Y24, Y41 in media of 275a.

30° ~~sh.~~ 11P21 Plate into T(0). 3P. 22.Growth ^(1:10) of ~~sup~~ plated Cells: elongated ^x/cell.

30°	1. Coli	+ 3	86^{21}	2×10^9	20	10^{-8}	+
	2. -glucose	+ 3	89	1.8×10^9	200	10^{-7}	
	3. -yx	+ 2	78^{21}	7×10^8	2	10^{-8}	
	4. pH variation	pH 8. → a b c d e f g h	78^{21} 72^{21} 73^{21} 85^{21}	3×10^9 4×10^9 3×10^9 2×10^9	100 40 30 10	3×10^{-8} 10^{-8} 10^{-8} 10^{-8}	{ + ++
	5. Buff x.	+ 3	79	3×10^9	5×10^3	10^{-6}	++++
	6. T(HCl)	+ 3	68	5×10^9	200	10^{-7}	++
	7. Malt Ex	? + seep-mater	1.2^{81}	6×10^8	0	0	-
	8. Coli hydrolys. T(0)	+ 3	79	3×10^9	10^4	10^{-6}	++++
9	10. 2% peptone	±	1.2^{93}	2×10^8	0		-
	11. Coli ^{var.} salt. a 10%	+4+	74	3×10^9	10	10^{-8}	+
	b 2%	+4+	82	3×10^9	10	10^{-8}	+
critical point?	c 5%	+4+	80	3×10^9	0	0	-
	d 10%	-					
	12. coli ^{unsh.}		93^2	1×10^8	10	10^{-7}	++
	13. coli ^{unsh.} + cegit.		86^2	1.5×10^8	1 - 10 ?	10^{-8}	+
	31. unsh. 20		95	10^9	10	10^{-8}	+
	32. 38°		95^2	10^8	0	0	-
	33. 10°		93	10^8	1	10^{-9}	±
	41. u.v.		86 (73 ²)	1.5×10^8	10	10^{-8}	+

[Salt inhibits recombination??]

Δ.

25-30° opt.
unsh.
-glucose.

inoculate 50 ml of the following media in Y24+Y41.
fresh.

30°

1. coli α (yx.3%; peptone.5% glucose.5%). See 276

2. yx.3% peptone.5% ~~glucose~~

3. Peptone.5%; glucose.5%

4. Peptone-yx-(glucose) in T(0) adjusted to various pH's.

5. Bactoextract-yx. broth.

.5%.

6. T(0) + N2ase + ~~in~~ VITS.

7. Malt extract 1%. (~~F~~ ~~in~~ ~~it~~).

8. T(0) + E. coli hydrolysate .1%.

9. Corn Yeast agar slants.

10. 2% peptone + biotin

~~31. coli~~ α 38°

32. coli α 38°

33. coli α 10°.

41. Irradiate 1 min. in u.v.
then into coli α 30° sl.

71. coli α + 1% NaCl 8 a
 2% NaCl 7 b
 5% NaCl 6 c
 10% NaCl 5 d
 4 e. no growth

1 2 3 4

conditions:

Optimal:

pH 7-8 } buffered
 - glucose
 - shaking
 low salt
 high nutrient N.
 25-30°

July 21, 1946.

In colis., 1030 P21 1 drop each of:

- ✓ ① Y10 x Y41
- ✓ 2. Y10 x Y24
- ✓ 3. Y41 x Y24
- 4. Y43 x Y41
- ✓ 5. D3 x Y41
- ✓ 6. D14 x Y41 agglutination!
- ✓ 7. D3 x Y43
- ✓ 8. D14 x Y43. agglut!
- ✓ 9. " Y43
- ✓ 10 Y41.

$$Y10 = TLB,$$

$$Y41 = TPR$$

~~T~~(0) T(B) T-1 (1st), etc. sacrifice.

PTR. B&C	1. B 2. B 3. B 4. B 5. O.	③	36 37 64 55 33
TLB, X B&C	11 O 12 B, 13 T 14 L 15 B 16 Ø 17 C 18 B, B 19 B, Ø 20 B, C 21 T, B 22 T, Ø 23 T, C 24 L, B 25 L, Ø 26 L, C	②	1 7 8 20 1 4 T 5 ++ 17 ✓ ++ 17 ✓ T 7 1 9 9 many 14 many 14 small 14 "

See 276

TPR	31.	O	(4)	O
AM	32	A		
	33	M	T	10^3
	34	T		15
	35	P		27
	36	AT		10^1
	37	AP		10^{-2}
	38	MT	T	10^3
	39	MP		10^3

Y43	41.	O	(9)	O
	42	O		0
	43	A		50
	44	M	T. ca 10^{-2}	100

Y41	51	O	(10)	O
	52	O	-	0
	53	T		100
	54	P		35

D3	Y41	61	O	(5)	T
		62	O	(6)	
		63	O	(7)	
		64	O	(8)	- hair-like threads -

microscopically, long bundles of filaments & large cells of varying length + sometimes broken up:



b. mycoides
see after

Segregation of vines - resistance (T-1)

278

23 JUL 1946

Use plates 4C, 5, and 8 of the cross B&C x PT₂. Test surface colonies directly for resistance to T-1 Sep. 275

4C.

5 R
13 S.

5

7 R
15 S

8

4 R
16 S

Total: 16 R / 60 total. Ca 25% recombination of R c either B+, T+ or P+

See 284. 5R/20.

Summary.

	one	16	60
284	5	20	
p.279	2	9	
			= 26%
284	23	89	
	5	21	
			28 110

found overgrowing?

Six conditions

23 JUL 1946

According to 275, a buffered meat-extract or coli-hydrolysate enriched medium is best for producing new prototrophs. Check on this with other nutrients.

Formula: ① Y41 + Y24 as 275.

② Y41 + SP-161

③ ~~Y41 + Y24~~ (which has yielded no prototrophic bacteria).
Y10 + Y43. $(R-1) \times B(R)$

Media: ~~Y~~ = "Yeast Buff broth = MxY
Ba = Bact. hydroly. 1mg/ml

PMx = Nutrient Broth.

Dose. 1 drop each (standard cultures). Incubate 30° (shaking).
1245 A 24. Plate 4P24.

Medium.	Dose.
1. MxY	①
2. MxY	2
3. PMx	1
4. PMx	2
5. T(PMx)	1
6. T(PMx)	2
7. MxY Ba	1
8. MxY Ba	2
9. MxY	3.

Results are not encouraging.

How different from 275? — time?
shaking?

7/24/46

See 279

For air conditions: Plate 4P24. (15h.)

$\frac{1}{1}$	$\frac{1}{1} \cdot 10^{-2}$	$\frac{100}{1}$	(100)
$\frac{2}{2}$	$\frac{1}{1} \cdot 10^{-2}$	$\frac{10}{0}$	10
$\frac{3}{1} \frac{2}{3}$	$\frac{1}{1} \cdot 10^{-3}$	$\frac{100}{1}$	(100)
$\frac{3}{2} \frac{2}{4}$	$\frac{1}{1} \cdot 10^{-3}$	$\frac{100}{1}$	(100)
$\frac{4}{1} \frac{2}{5}$	$\frac{1}{1} \cdot 10^{-2}$	$\frac{100}{0}$	100
$\frac{5}{1} \frac{2}{6}$	$\frac{1}{1} \cdot 10^{-2}$	$\frac{200}{2}$	(200)
$\frac{6}{1} \frac{2}{7}$	$\frac{1}{1} \cdot 10^{-2}$	$\frac{50}{0}$	50
$\frac{7}{1}$	$\frac{1}{1} \cdot 10^{-2}$	$\frac{10}{0}$	
$\frac{8}{1}$	$\frac{1}{1} \cdot 10^{-2}$	$\frac{10}{0}$	
$\frac{9}{1}$	$\frac{1}{1} \cdot 10^{-2}$	$\frac{0}{0}$	

For recombination types:

1030P24

② 10^{-3}

1	O
2	B
3	M
4	P
5	T
6	MP
7	MT
8	BP
9	BT
10	O
11	O
12	O

for delicate
n.g.

① 10^{-3}

21	O
22	O
23	O
24	B
25	B
26	B

K-12 x B/1.

282

11P24. 1ml Y41 + Y43 in YB. 25. 30°

N28. Drop into T(0) plates.

O

to 7P25, on desktop. Backs on shelves.

1P27. Plate out. O

Y43 x Y44

O

11P24. Inoc YB D14, Y41 Sh 30°

10A25. Inoc YB 1 ml each of above Sh 30°

to A27. Only typical bacilli.

[Repeat in yeast ext - peptone medium]

P27 Repeat in colo.

a) D14 + Y41 - only bacilli

b) agar plate only atypical forms \rightarrow

(actinomycete?) But has filaments of long cells like *Sphaerotilis*, staining well w/ safranin.

Isolate + determine nutr. req., drug resistance, to exclude origin. (completes [how about *Proteus x coli*?]) Strains not form supernatant after larger masses have settled.

Grows on plate like filamentous fungus. Refer to 283

B. mycoides according Tatum